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MINIREVIEW

TRH-like Peptides in Prostate Gland and other Tissues

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Summary

This minireview is aimed to recapitulate the occurrence of TRH-like peptides in the prostate gland and other tissues and to discuss their known functions in the organism. The hypothalamic thyrotropin-releasing hormone (TRH) was the first chemically defined hypophyseotropic hormone with the primary structure pGLU-HIS-PRO.NH_2. However, the presence of extrahypothalamic TRH-immunoreactive peptides was reported in peripheral tissues including the gastrointestinal tract, placenta, neural tissues, male reproductive system and certain endocrine tissues. It was supposed that this TRH immunoreactivity can partially originate from TRH-homologous peptides and that these peptides have significant cross-reactions with the antibody specific against authentic TRH. This assumption was confirmed by the identification of prostatic TRH immunoreactivity as pyroGLU-GLU-PRO.NH_2 using fast atom bombardment mass spectrometry and gas phase sequence analysis. TRH-like peptides are characterized by substitution of the basic amino acid histidine (related to authentic TRH) for neutral or acidic amino acids, such as glutamic acid, phenylalanine, glutamine or tyrosine. The physiological role of TRH-like peptides in peripheral tissues is not precisely known, but they possess a C-terminal amide group which is characteristic for many biologically active peptides. The occurrence of these peptides in the male reproductive system can influence male fertility. They are also closely related to circulating thyroid and steroid hormones. There might be an important connection of TRH-like peptides to the prostatic local autocrine/paracrine network mediated by extrahypothalamic TRH immunoreactivity corresponding to TRH-like peptides and extrapituitary thyrotropin (TSH) immunoreactivity also found in the prostatic tissue. A similar system of intraepithelial lymphocyte hormonal regulation due to the local paracrine network of TRH/TSH has been described in the gastrointestinal tract. The local network of TRH-like peptides/TSH may be involved in possible regulation of prostatic growth.

Key words
TRH • TRH-like peptides • Prostate • Extrahypothalamic occurrence • pEEPam • pEFPam

Introduction

The history of TRH-like peptides is closely linked to the history of thyrotropin-releasing hormone (TRH), a tripeptide (pyroglutamyl-histidinyl-prolin amide, pGLU-HIS-PRO.NH_2, pEHPam), which is a major hypothalamic factor in thyrotropin (TSH) secretion. TRH was the first hypophyseotropic hormone, which was chemically defined. Its discovery in 1969 by Guillemin, Schally and their coworkers was crucial for

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the validation of the hypophyseal-portal vessel-chemotransmitter hypothesis of pituitary control (Reichlin 1992). It should be mentioned here that Professor V. Schreiber already in 1959 reported that the activity of acid phosphatase in animal anterior pituitary glands is increased after their incubation with the hypothalamic extract. Since this activity in the pituitary rose markedly after thyroidectomy, he formulated the hypothesis that adenohypophyseal acid phosphatase is related to TSH secretion and that TRH is its possible activator (Schreiber and Kmentová 1959a, b).

Four TRH-like peptides, which have so far been identified, are characterized by having their basic amino acid histidine (related to authentic TRH) exchanged for neutral or acidic amino acid (glutamic acid, phenylalanine, glutamine or tyrosine). The primary structures of TRH-like peptides are: pyroGLU-GLU-PRO.NH_2 (pEEPam), pyroGLU-GLN-PRO.NH_2 (pEQPam), pyroGLU-PHE-PRO.NH_2 (pEFPam) and pyroGLU-TYR-PRO.NH_2 (pEYPam). TRH-like peptides cannot be expressed from the TRH gene and up to now it is not known which gene is responsible for the expression of these peptides. Only a part of the semenogelin molecule from the male reproductive tract has the sequence of TRH-like peptide pEEPam (Lilja 1990), but this protein is not the precursor of pEEPam (Huber *et al.* 1998). The three-dimensional structures of TRH and probably the main TRH-like peptide – pEEPam are shown in Figure 1.

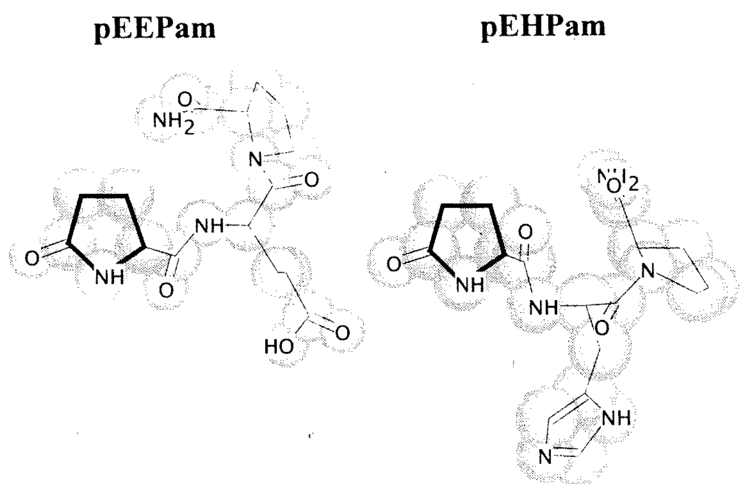

Fig 1. *Three-dimensional structure of TRH-like peptide pyroGLU-GLU-PRO.NH_2 (pEEPam) and TRH (pyroGLU-HIS-PRO.NH_2, (pEHPam)*

The occurrence of extrahypothalamic TRH immunoreactivity

One of the first reports concerning the extrahypothalamic occurrence of TRH immunoreactivity was published by Schaeffer *et al.* (1977) who described that the material from the rat retina reacts with an antibody specific for TRH. The immunoreactivity present in the retina was co-chromatographed with authentic TRH. In the same year was demonstrated that TRH is present in the skin of the frog (*Rana pipiens*) in concentrations twice as high as those found in the hypothalamus of this species (Jackson *et al.* 1977). One year later, TRH immunoreactivity was found in rat islets of Langerhans (Martino *et al.* 1978). Moreover, the distribution of TRH immunoreactivity was described in rat neural tissues (hypothalamus, spinal cord, medulla, cerebrum and cerebellum), rat gastrointestinal tract (pancreas, stomach, duodenum and colon) (Leppaluoto *et al.* 1978) as well as in rat pancreas and eye, bovine and sheep pineals, and human placenta (Youngblood *et al.* 1979). Similar results were obtained by Pekary *et al.* (1983b) who demonstrated the presence of TRH immunoreactivity in various canine tissues. Using TRH radioimmunoassay, SP Sephadex C-25 cation exchange chromatography, Sephadex G-10 exclusion chromatography and high pressure liquid chromatography (HPLC), these authors were able to differentiate between authentic hypothalamic TRH and TRH-like peptides that were present in the liver, adrenals, pancreas, thyroid gland, prostate, epididymis, testis and semen. The other study on the distribution of TRH and TRH-like peptides in the rat (Del Rio-Garcia and Smyth 1990) indicated that TRH-like peptides in the brain were located principally in the hippocampus, brain stem and dorsal colliculi. In the periphery, TRH-like peptides were present in the male reproductive system and certain endocrine tissues. The pyroglutamyl-glutamyl-proline amide (pEEPam) was

found in the rat anterior and posterior pituitary gland after its separation from authentic TRH by anion-exchange chromatography and only trace levels of this pEEPam were observed in the hypothalamus (Ashworth *et al.* 1991a). Ashworth *et al.* (1991b) also reported that pEEPam was present in the porcine pituitary but was absent in the porcine reproductive tract, where could be of biological significance. Concerning the rat thyroid gland and hypothalamus, Rausell *et al.* (1999) described the presence of authentic TRH, pEEPam and pEFPam (pyroglutamyl-phenylalaninyl-proline amide) in the rat thyroid gland. The concentrations of TRH-like peptides were much higher in hyperthyroid than in hypothyroid rats. Similar results were obtained by Smyth *et al.* (1999) who found that the human thyroid gland contains both authentic TRH and TRH-like peptides and a similar pattern was found in several animal species. Mendez *et al.* (1999) have suggested that the pEFPam and other TRH-like peptides are present in the rat hypothalamus, because they found three TRH immunoreactive peaks (by means of HPLC followed by TRH radioimmunoassay) in the medium and tissues of rat hypothalamic slices. It is interesting that Jackson (1981) reported a high TRH immunoreactivity in extracts of a plant, alfalfa. This TRH immunoreactivity was subsequently purified by a combination of exclusion, anion and high pressure chromatography and was identified using gas phase sequencing and fast atom bombardment mass spectrometry (FAB MS) as pyroGLU-TYR-PRO.NH_2 (Lackey 1992).

It is not clear, whether the TRH immunoreactivity found in various extrahypothalamic tissues is the result of cross-reactions between the TRH specific antibody and small peptides or substances present in the biological matrix, or if it is due to authentic TRH and TRH-like peptides. The evidence that the rat pancreas is able to synthesize TRH, was reported by Theodoropoulos and Zolman (1985). They studied the *in vitro* synthesis of TRH by neonatal pancreatic cells, using [^{14}C]histidine incorporation following Sephadex G-200 chromatography with synthetic TRH as a marker. The eluted radioactivity together with TRH immunoreactivity was subsequently determined in the elution profile by specific TRH radioimmunoassay. Dutour *et al.* (1987) isolated pancreatic RNAs and Northern blot preparations were hybridized with ^{32}P-labelled TRH cDNA probes. They thus detected pancreatic TRH mRNA. A fast atom bombardment mass spectrometric method combined with HPLC was used for the identification of authentic TRH in secretions from the skin of frog (*Xenopus laevis*) (Gibson *et al.* 1986). Gkonos *et al.* (1989) investigated whether TRH gene expression also occurs in normal rat thyroid parafollicular cells. Northern analysis of total thyroid RNA with a preproTRH-specific RNA probe demonstrated authentic TRH gene expression in these cells.

TRH-like peptides in male reproductive tract

From our point of view, the results of Pekary *et al.* (1980) concerning the presence of TRH and a homologous peptide in the male rat reproductive system are important. Pekary *et al.* (1983a) supposed that TRH immunoreactivity found in human semen and in the rat or human prostate has the general structure pyroGLU-X-PRO.NH_2, where X is either basic histidine (authentic TRH) or a neutral amino acid (TRH-like peptides). Using FAB MS and automatic gas phase sequence analysis, Cockle *et al.* (1989a) identified a prostatic peptide with TRH immunoreactivity from the rabbit prostate complex as pyroGLU-GLU-PRO.NH_2 whereas pyroGLU-GLU-PRO.NH_2 was found in human semen (Cockle *et al.* 1989b). A similar procedure (HPLC, FAB MS, amino acid analysis) was used for the identification of TRH immunoreactivity in human seminal fluid (Khan *et al.* 1992) where TRH-like peptides with primary structures pyroGLU-PHE-PRO.NH_2 or pyroGLU-GLN-PRO.NH_2 were detected. These findings were supported by Gkonos *et al.* (1994) who identified the pEFPam in normal human and rat prostate by ion-exchange chromatography and reversed-phase HPLC. Bílek *et al.* (1992) examined the secretory pattern of prostatic TRH immunoreactivity. After separation of TRH immunoreactivity from the methanolic extract of rat prostate glands into two groups by minicolumn cation-exchange chromatography, the amount of peptides were quantified by anti-TRH specific radioimmunoassay. The acidic or neutral TRH-like peptides unretained on the minicolumn and the retained fraction of authentic TRH containing the basic histidine were further separated by reversed phase HPLC. The amount of TRH immunoreactivity was determined in the collected fractions. The unretained fraction was found to contain a series of TRH-immunoreactive peptides corresponding chromatographically to pEEPam or pEFPam. None of the TRH-immunoreactive peptides exhibited the chromatographic behavior of TRH. The absence of TRH gene expression in the prostate was also

demonstrated by Northern blot analysis and by application of polymerase chain reaction amplification, which failed to reveal TRH mRNA. Linden *et al.* (1996) assumed that TRH-like peptides in the rabbit testis are different from the TRH-like peptide in the prostate after trypsin digestion. In contrast to the prostate, the testis contained high concentrations of N-extended forms of pEFPam, but essentially no tripeptide. The same results concerning TRH immunoreactivity in the human testis and prostate were found in our study (Bílek *et al.* 1994). Montagne *et al.* (1996) also found three TRH immunoreactive peptides in the rat testis. The two major forms exhibited the same chromatographic properties as synthetic TRH and pEFPam. These peptides were only detected after ethylene dimethanesulfonate treatment of adult rats, which was followed by Leydig cell destruction. After the regeneration of Leydig cells, all three TRH-immunoreactive substances were detectable in trace amounts only.

The regulation and a possible biological function of TRH-like peptides

One of the first studies (Gordeladze *et al.* 1988) indicated that TRH stimulates the adenylyl cyclase in normal (basal cells), hyperplastic and adeno-carcinomatous prostate as well as in the pituitary gland and the stomach. However, Ashworth *et al.* (1994) reported that both TRH and pEEPam act through the same intracellular signaling system, causing a significant increase of intracellular inositol phosphate in GH3 cells (anterior pituitary cells in culture). Harvey *et al.* (1993) demonstrated that pEEPam may act as a TRH receptor antagonist in the avian hypothalamo-pituitary system. Thyroid hormones can modulate TRH biosynthesis in rat extrahypothalamic tissues (Simard *et al.* 1989, Mitsuma *et al.* 1990), but the pathological conditions, such as altered thyroid and adrenal status, or suckling did not affect the levels of pEEPam in the rat anterior pituitary gland (Rondeel *et al.* 1995). Cremades *et al.* (1998) described *in vivo* activities of TRH-like peptides. Both pEEPam and pEFPam were able to influence the thyroid status, *i.e.* to increase T_3 and to a lesser extent T_4 in the circulation, if they were administrated to mice. pEFPam was more potent than pEEPam with a similar potency to TRH. A significantly greater effect was produced in females than in males. Castration of male mice increased the activities of TRH-like peptides, whereas treatment of female mice with testosterone reduced the activity of these peptides. The evidence that TRH-like peptides are dependent on androgens were also published by other authors. Rondeel *et al.* (1995) mentioned a distinct sex difference in the concentration of pEEPam in the rat anterior pituitary. Levels of pEEPam were higher in male than in female rats. In both sexes, gonadectomy caused a substantial two- to threefold rise in pEEPam concentrations. Testosterone administration normalized the levels of pEEPam. However, Thetford *et al.* (1992) found that the concentration of rabbit prostatic pEEPam was increasing with the age of animals. At eleven weeks of age, the levels of TRH-immunoreactive peptides were very low, but considerably higher concentrations were found in animals older than 4 months. The influence of dexamethasone on the concentration of pEEPam in the rat pituitary was described by Akinsanya *et al.* (1995). Dexamethasone treatment resulted in a twofold increase of pEEPam in the rat anterior pituitary. Cockle *et al.* (1994) and Green *et al.* (1994) have named the pEEPam as a fertilization-promoting peptide because of its ability to enhance the *in vitro* fertilizing potential of mouse epididymal spermatozoa. The influence of TRH-like peptides on pancreatic tissues was also observed. Kulkarni *et al.* (1995) reported the influence of pEFPam on isolated perifused rat islets and glucose-responsive clonal cell lines and concluded that TRH potentiates, whereas pEFPam inhibits glucose-stimulated insulin release in these islets and cell lines. pEFPam also reversed the stimulatory effect of TRH. High serum levels of pEEPam were observed in patients with carcinoid tumors. The mechanism of pEEPam production by these tumors and its possible biological function still remain unknown (Klootwijk *et al.* 1996). Farnsworth (1993) discussed the participation of prolactin in prostatic physiology. A novel aspect was introduced by this author who mentioned the presence of androgen- and prolactin-dependent concentration of TRH immunoreactivity in the prostatic tissue. It was hypothesized that TRH may serve as a mediator of prolactin-independent and androgen-dependent control of the growth and function of the prostate gland.

The results of our recent experiments have shown that the mature rat prostate does not contain authentic TRH, but that TRH-like peptides are present. However, the concentration of these peptides is sensitive to thyroid hormone administration (Bílek *et al.* 1992). The rat prostate concentration of TRH-like peptides was

significantly elevated by castration, while testosterone treatment of castrated rats restored the normal levels of TRH-like peptides (Bílek *et al.* 1991, 1996). Our unpublished results obtained in patients with prostatic carcinoma and benign prostatic hyperplasia revealed interesting correlations, particularly between thyroxine (T_4), triiodothyronine (T_3) and prostatic specific antigen (PSA), or between testosterone and T_4 or T_3. It is evident that there is some relationship between thyroid hormones and the prostate gland. This relation seems to be enhanced by TSH and TRH immunoreactivity found in the prostate. TSH has a close relationship with TRH immunoreactivity, which positively correlates with calcitonin immunoreactivity. The prostatic TSH immunoreactivity was negatively associated with serum T_3 in our experiments. The presence of TSH-like peptides in the prostate gland was demonstrated in neuroendocrine cells after analysis of human prostate tissue homogenates with SDS-PAGE followed by immunoblotting (Abrahamsson and Lilja 1989). The prostatic TSH may exert stimulatory effects on the thyroid gland in rats (Mani Maran *et al.* 1998). In the case of calcitonin, its receptor expression in the prostate is located in subsets of dispersed neuroendocrine cells. This indicates that prostate calcitonin may play an important role in the autocrine /paracrine regulation of the prostate (Wu *et al.* 1996). The relationships between extrahypothalamic TRH immunoreactivity, pituitary/extrapituitary TSH, and thyroid/extrathyroid calcitonin open new possibilities for explaining the physiological processes in the prostate gland.

Conclusions

The human prostate is a complex system composed of mesenchymal tissue which contains exocrine basal and luminal cells, and epithelial tissue lined with neuroendocrine cells (Xue *et al.* 1998, Lobaccaro *et al.* 1997). It is known that the main hormonal influence acting upon the prostate is exerted by androgens. It is commonly accepted that the androgen effect is followed by pathological changes in the prostate. Nevertheless, androgens alone are not sufficient for inducing normal or pathological growth and function of the prostate (Reiter *et al.* 1999). For example, approximately one week after orchidectomy, atrophic changes have been reported in the prostatic epithelium but, on the contrary, the interstitial prostatic tissue hypertrophies (Wahlqvist *et al.* 1996). This means that all subsequent changes taking place in the prostate are more complex and not yet fully understood. The decline of testicular function introduces a dysbalance into the paracrine or autocrine production of various growth factors influencing the prostate. It seems likely that androgens exert their influence on the prostate by regulating the synthesis of growth factors. It could be supposed that a sufficient level of androgens is able to suppress the production of prostatic growth factors, and *vice versa.* Following imbalance, the synthesis of growth factors is induced. This seems to be compatible with the fact that the number of patients with benign or malignant prostatic disease increases with age and it is accompanied with a decline of androgens. It is questionable which peptides or proteins with growth factor properties participate in prostatic growth regulation. The discussed growth factors include, for example, insulin-like growth factors (Lobaccaro *et al.* 1997, Monti *et al.* 1998) or the family of fibroblast growth factors (Cussenot 1997), both occurring in the prostate.

A new insight into the role of TRH (thyroliberin) and TSH (thyrotropin) were recently described (Shanahan 1997, Wang *et al.* 1997). These authors presume a local paracrine network of intraepithelial lymphocyte hormonal regulation in a gastrointestinal tract. Extrahypothalamic TRH reacts with receptors on enterocytes, which is followed by TSH expression. TSH is bound to specific receptors on intraepithelial lymphocytes. It may be suggested that this role of TRH could be overtaken by prostatic TRH-like peptides, particularly when the endocrine cells of prostate produce TRH- and TSH-like peptides. The prostatic gland is affected, most commonly, by benign prostatic hyperplasia or prostatic cancer. They pose difficult medical and social-economical problem regarding their high prevalence among men over fifty years old. The local network of TRH-like peptides–TSH may bring new possibilities of regulation mechanisms in prostatic growth.

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